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CONTINUATION

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MORPHATIDES: NOVEL SHAPE AND STRUCTURE LIBRARIES

by

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MORPHATIDES: NOVEL SHAPE AND STRUCTURE LIBRARIES

Sub A2

This application is a continuation-in-part of U.S.S.N. 08/953,634 filed October 17, 1997, which is a continuation-in-part of U.S.S.N. 08/839,468 filed April 14, 1997, and claims the benefit of copending U.S. Provisional Application Serial No. 60/028,527 filed October 17, 1996.

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

BACKGROUND OF THE INVENTION

The present invention relates to the production and screening of libraries of compounds and, more particularly, to the generation and screening of shape and structure libraries produced from large or small size molecules for the purpose of identifying potentially useful agents.

New agents for effectively modulating a range of biological processes have a variety of applications in industry, medicine and agriculture. The identification of structurally unique lead compounds is an important step in selecting such biologically useful agents. Historically and currently, mass screening of collections of large numbers of molecules (chemicals or other compounds) and mixtures of molecules, has been the most successful approach for identifying lead compounds. Most of these collections are either compound databases generated by pharmaceutical research, natural products collections, such as fermentation broths, or more

recently, collections of peptides, nucleotides or other synthesized molecules.

Each of these collections, or libraries, has its advantages as well as its limitations. Collections generated via research, such as compound databases, can obtain a potentially limitless repertoire of compounds for search (large numbers), however they tend to contain a limited number of diverse structures, representing only a small portion of the total structural diversity possibilities. Natural product libraries can offer structural complexity, however the difficulty in downstream manufacturing of these products, and of reducing leads to useful products is a serious limitation of this type of approach. Peptide libraries are limited to peptides or peptide mimics. There has been limited success in the conversion of peptide chemical leads into pharmaceutically useful drug candidates. These lead compounds are at a disadvantage for generating orally active drug candidates due to the complexity of determining their three dimensional structures for synthesis of small organic molecules, and due to the sensitivity of their peptide bonds to acid hydrolysis. However, the structural diversity offered by this technology is its greatest advantage. Nucleotide libraries are also restricted to the genetic repertoire (nucleotides) or nucleotide analogues that preserve specific Watson-Crick pairing and can be copied by a polymerase, hence they are more limited in their useful structural diversity than peptide libraries, however this remains an advantage of these libraries. Nucleotide libraries also offer the capacity for cloning and amplification of DNA sequences, which allows for enrichment by serial selection and provides a facile method for decoding the structure of active molecules.

Compound databases have historically been generated via the chemical modification of existing compounds to generate analogs, which then follow the conventional paradigm of small molecule lead

development in which a compound undergoes many rounds of individualized, hand-crafted modification and biological testing en route to drug candidacy. Natural product libraries are derived from collections of natural materials, such as fermentation broths, plant extracts, etc.

Peptide and nucleotide libraries are generated by sequence randomization of individual monomers using a single naturally existing biological linkage (3'-5' phosphate linkage of nucleotides or amide linkage of peptides). As indicated, the biggest advantage in using peptide and nucleotide libraries is the apparent structural diversity afforded with the technologies. For example, Figure 1 briefly demonstrates one well known strategy for generating and utilizing Aptamers, a library of nucleotide shapes.

For the discovery of drugs and other commercially valuable compounds, small molecule, highly complex libraries containing diverse functionalities have the greatest utility and provide the greatest chance of success. Libraries must also permit identification and evaluation of the structure/activity relationship of the potentially small fraction of active molecules among the larger number of inactive or less active compounds. To satisfy these needs, recent trends are to generate chemical libraries and new techniques to evaluate and screen them. Chemical libraries have been defined as intentionally created collections of differing molecules which can be prepared synthetically or biosynthetically. A type of synthetic strategy which can lead to large chemical libraries is combinatorial chemistry. Combinatorial chemistry has been defined as the systematic and repetitive, covalent connection of a set of different 'building blocks' of varying structures to each other to yield a large array of diverse molecular entities. (Gallop, M.A. et al., 1994) Building blocks can include nucleotides, carbohydrates, peptides or peptoids into ordered structures. Chemical libraries generated utilizing

combinatorial chemistry can display remarkable diversity. These large libraries can be selected for potential pharmacological activity by their affinity to specified ligands. Several groups have taken advantage of these facts to develop systems utilizing modified and unmodified oligonucleotides and modified and unmodified polypeptides as ligands to bind targets. Many examples are available in the art, a few of which are described herein.

Although oligonucleotides inherently have fewer potential building blocks to provide diversity, they have demonstrated a remarkable affinity for selected targets. Examples include both single-stranded RNA and single- and double-stranded DNA. A great attraction of nucleic acid based combinatorial chemistry is the potential for directed evolution. Repeated cycles of selection for the highest affinity and error-prone PCR can lead to increased diversity and oligomers with an even greater affinity.

The versatility of the binding capabilities of DNA and RNA oligonucleotides seems inexhaustible and the growing number of applications are a tribute to its enormous potential. One of the earliest polynucleotides of this type was directed to the human blood clotting enzyme thrombin (Bock et al., 1992). This study initiated a search for other thrombin inhibitors based on this approach (Bracht and Schroer, 1994; Kubik et al., 1994; Griffin et al., 1993). Numerous other polynucleotide sequences have been selected from initially random libraries of molecules. Examples include DNA and RNA oligomers selected against HIV integrase (Allen et al., 1995), its Rev protein (Giver et al., 1993; Tuerk and MacDougal-Waugh, 1993; Jensen et al., 1994; Jensen et al., 1995; Bartel et al., 1991), and its reverse transcriptase (Chen and Gold, 1994; Tuerk et al., 1992; Schneider et al., 1995), as well as against reverse transcriptase of feline immunodeficiency virus (Chen et al., 1996). Other were developed against human growth factors, such as nerve GF (Binkley et al., 1995), vascular

endothelial GF (Jellinek et al., 1994), and basic fibroblast GF (Jellinek et al., 1996; Jellinek et al., 1995) and against Qβ replicase (Brown and Gold, 1995a; Brown and Gold, 1995b). An RNA oligomer has been made against nucleolin (Ghisolfi-Nieto et al., 1996), an essential protein in ribosome biosynthesis. Oligomers against selectins may show potential in treatment of anti-inflammatory diseases (O'Connell et al., 1996). Other proteins against which these oligomers have been selected include immunoglobulin IgE (Wiegand et al., 1996), bacteriophage T4 DNA polymerase (Tuerk and Gold, 1990), bacteriophage R17 coat protein (Schneider et al., 1992), the E. coli rho factor (Schneider et al., 1993), leucine receptive regulatory protein (Cui et al., 1995) and several ribosomal proteins (Dobbelstein and Shenk, 1995; Ringquist et al., 1995).

These polynucleotides can also be selected for their affinity to small molecules. These include early experiments which demonstrated RNA oligomers that bind specifically to a variety of dye molecules (Ellington and Szostak, 1990). Later this finding was extended to DNA oligomers (Ellington and Szostak, 1992). Interestingly, the sequences of these DNA and RNA species are quite distinct, even when selected for the identical substrates.

The oligopeptide substance P, a mammalian neuro-transmitter, was used to select RNA molecules with high affinity against the neurotransmitter by Nieuwlandt *et al.* (1995). Single amino acids and other small molecules are also able to bind such molecules. Examples include valine (Majerfeld and Yarus, 1994), arginine (Yarus and Majerfeld, 1992; Puglisis *et al.*, 1992; Nolte *et al.*, 1996; Hicke *et al.*, 1989; Geiger *et al.*, 1996; Burgstaller *et al.*, 1995), citrulline (Burgstaller *et al.*, 1995), ATP (Huizinga and Szostak, 1995; Sassanfar and Szostak, 1993), adenosine (Huizinga and Szostak, 1995), D-adenosine (Kluszmann *et al.*, 1996), flavin

mono-nucleotide (Fan *et al.*, 1996), theophylline (Jenison *et al.*, 1994), cyanocobalamine (Lorsch and Szostak, 1994).

Another interesting potential of these oligomers was pursued by Morris *et al.* (1994) who tried unsuccessfully to select for a molecule specific for a reaction transition state, effectively attempting to create a catalyst. Hale and Schimmel (1996), however, did succeed in generating a DNA molecule that induces hydrolysis of a misactivated amino acid bound to a tRNA synthetase, a case of protein synthesis editing. Lorsch and Szostak (1994) succeeded in selecting for several RNA aptamers with 2' or 5' polynucleotide kinase activity.

Polynucleotides with modifications of incorporated nucleotides have been selected by Latham *et al.* (1994), who incorporated 5-(1-pentynyl)-2'-deoxyuridine into thrombin binding DNA molecules. The primary sequence of these modified DNA oligomers was strikingly different from the unmodified DNA molecule.

The use of nucleic acids for therapeutic and diagnostic applications often requires their stability in biological fluids. Aside from chemical modification, nuclease-resistant ligands can be generated by using L-ribose-based nucleotides (Nolte *et al.* 1996, Klussmann *et al.* 1996). In this approach the conventional D-RNA directed against the optical mirror image of the target is selected first using repeated rounds of mutation and selection of the nucleic acid and subsequently the corresponding L-RNA is chemically synthesized. L-RNA's with specificity for L-arginine (38-mer, Kd=60 mM, Nolte *et al* 1996) and D-adensosine (58-mer, Kd=1.7 mM, Klussmann *et al.* 1996) have been isolated and shown to be stable in human serum at 37°C. Another example includes chirally pure methylphosphonate linkages that are suitable for generating oligomers capable of efficiently hybridizing with DNA or RNA and

are highly resistant to metabolic breakdown in biological systems (Reynolds *et al.* 1996).

Another interesting method for the selection of nucleic acid molecules with highly specific binding to target molecules has been developed and termed "SELEX" (Systematic Evolution of Ligands by EXponential enrichment), which is described in U.S. Patent No. 5,270,163 entitled "Nucleic Acid Ligands" and in PCT/US91/04078. SELEX is a method for making a nucleic acid to a desired target molecule involving the selection from a mixture of candidate oligonucleotides and the step-wise iteration of binding, partitioning and amplifying, using the same general selection scheme, to achieve a desired criterion of binding affinity and selectivity. The basic SELEX method has also been modified to achieve a number of specific objectives. (For instance, those described in PCT/US94/10562 filed September 19, 1994, and WO 96/09316 filed September 19, 1995).

For example, SELEX has been used in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA; as a method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photo-crosslinking to and/or photoinactivating a target molecule; in the identification of certain nucleic acid sequences that contain 5-iodouracil residues and that covalently bind to HIV-1 Rev protein; in the identification of highly specific nucleic acid ligands able to discriminate between the closely related molecules, theophylline and caffeine; as a method to achieve efficient partitioning between oligonucleotides having high and low affinity for a target molecule; and as a method for covalently linking a nucleic acid to its target.

The SELEX method relies on a process of selection and amplification for enrichment of desired candidate positives from a collection of candidates to identify better candidates or the best candidates from the collection. During the selection part of the process from each parent collection, the bulk binding of the populations of candidates becomes increasingly higher as the sequences are amplified, and those sequences unable to interact with the target are eliminated from the population. Hence, "evolution" of the population occurs due to the increased presence due to amplification of candidates which exhibit the desired activity and the effective elimination of undesirable candidates. Amplification is used to increase the presence of desirable products and to separate those products from those that do not react or have a weaker reaction with a target of interest. (WO 96/09316 entitled Parallel Selex)

The Parallel SELEX method describes one potential technique for the identification of DNAs that have facilitating activities as measured by their ability to facilitate formation of a covalent bond between the DNA, including an associated functional unit, and its target. Although this method focuses on the facilitative binding capabilities of DNA, it does not take advantage of the potential for nucleic acids to be evolved in vitro via methods such as Error-prone PCR or Sexual PCR. The method defines the pool, or collection, of DNAs as being evolved due to the enrichment of positives that occurs via an amplification reaction (exponential enrichment). The DNA molecules themselves are never evolved.

SUMMARY OF THE INVENTION

There currently exists need for novel systems which combine the advantages of screening the different types of collections mentioned; one which allows the enrichment by serial selection and facilitates the decoding of the structure of lead candidates

afforded by screening nucleotides, and which simultaneously provides the potentially limitless repertoires of diverse molecules for screening offered by chemical compound and natural product collections. The present invention provides a novel approach for creating diverse, complex shape and structure libraries of large or small size agent molecules and for screening said libraries to identify compounds having a wide variety of commercially valuable industrial applications. Not only does the present invention provide a limitless repertoire of diverse structures that may be screened for biological activity, but it provides an iterative selection and enhancement process to define the most active compounds, and it is a process that allows one to solve the structure (if desired) of the most active compounds rapidly. These processes for mutating and selecting compounds to effectively "evolve" chemical groups in order to identify a most useful compound(s) and the ability to rapidly solve the structure of identified compounds are significant advantages of the present invention. These advantages and other features distinguish the present invention from previously existing technologies.

The present invention provides a method for identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; (ii) one or more linker components; and (iii) one or more agent molecules or type of agent molecules, linked to the scaffolding component by one or more type of linker components; and (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or

more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure; (c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure.

The present invention also provides a method for identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; and (ii) one or more agent molecules or type of agent molecules, associated, bound, or bonded to the scaffolding component; (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target

molecules through the preselected structure; (c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a preselected structure.

The present invention further provides a method of identifying a presence of a substance in a sample from a subject, comprising: (a) obtaining a sample; (b) contacting the sample with one or more types of morphatide identified by either of the methods for identifying one or more complexes so as to form a complex between the morphatide and the substance present in the sample; (c) detecting the complex formed in step (b), thereby identifying the presence of the substance in the sample from the subject.

This invention further provides a method of diagnosing a subject wherein detection of a complex in step (c) of a method of identifying a presence of a substance in a sample is indicative of a disease.

Another aspect of the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with multiple types of molecules. Another aspect of the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with one type of molecule.

Still another aspect of the present invention provides a composition comprising the preferred embodiment of the morphatide effective to treat a subject and a pharmaceutically acceptable carrier.

The present invention further provides a morphatide labeled with a detectable marker.

This invention also provides a method of treating a subject with compositions of morphatides and morphatides conjugated to therapeutic agents.

In another aspect, this invention provides a method of drug delivery to a target in the body of a subject comprising administration to a subject any of the above-described compositions of morphatides and morphatides conjugated to therapeutic agents.

This invention further provides a method of drug delivery to a target in the body of a subject comprising administration to a subject of the above-described compositions of morphatides and morphatides conjugated to therapeutic agents, wherein the morphatide is incapable of being degraded or is slowly degraded after administration to the subject, thereby delivering the morphatide-bound drug to the target.

This invention still further provides a morphatide that is capable of binding to any component of an antibody molecule, said antibody having a constant and variable region.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Briefly demonstrates one well known strategy for generating and utilizing Aptamers, a library of nucleotide shapes.

Figures 2A-B. Present an example of the method described in the present invention beginning with a template nucleic acid molecule as the scaffolding molecule.

Figure 3. Depicts a similar example of the method described in the present invention, indicating the target molecules bound to a solid support, and the dissociation of the complex molecules from the target molecules occurring via elution with a pH shift.

Figure 4. Depicts the use of a particular type of bioconjugate, phenyl boronic acid, which can be used as a linker in the present method.

DETAILED DESCRIPTION OF THE INVENTION

Unlike previously described technologies and methods, the present invention is an approach that maintains the facile ability of nucleic acids to evolve, while introducing the properties of additional components to create molecules with binding properties previously restricted to protein-like molecules. The basis of this invention is a Morphatide (previously termed Morphotide). A morphatide, as used herein, is a complex comprised of a scaffolding component, hereinafter defined, one or more linker components, hereinafter defined, and one or more agent molecules, hereinafter defined. Once desirable Morphatides are identified, scaffolding components can be separated from the agent molecules, evolved to generate a new, different and potentially better library of scaffolds, reconnected to the same or different agent molecules to generate a new library of Morphatides, and rescreened for an even more desirable activity, of either the entire Morphatide or individual components thereof. This approach permits the directed evolution of polynucleotide molecules which can be disconnected, amplified and evolved. The present invention is thus an approach which couples the distinct advantage of the self replication of oligonucleotides and their potential to be evolved, with the

richness of diversity of chemical modifications hitherto associated with other types of libraries.

In accordance with the present invention, libraries comprising structural or scaffolding components, linker molecules, and agent molecules are produced. Alternatively, the libraries may comprise structural or scaffolding components and agent molecules without linker molecules. Useful candidate compounds are identified from said shape and structure libraries. The useful candidate compounds can then be separated from the library and either modified to generate an even more useful candidate, or utilized directly.

The novel shapes and structures generated utilizing the present invention are named Morphatides. The process of generating and screening a Morphatide library is deemed Morphatide based combinatorial chemistry. Morphatide combinatorial chemistry can begin with a template scaffolding molecule. In one example, if this scaffolding molecule is a nucleic acid molecule, the molecule is amplified utilizing a process known as sloppy PCR, error-prone PCR or mutagenic PCR (PCR Primer, A Laboratory Manual, Cold Spring Harbor Laboratory Press 1995) and nucleotides that have been coupled to, associated with, or attached to a component of a linker molecule or to a linker molecule. The nucleotides can be naturally occurring, novel or unique, or nucleotide analogs. Error-prone, or sloppy or mutagenic, PCR is a process for performing the polymerase chain reaction under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. This process generates various scaffolding molecules with components of linker molecules or linker molecules randomly attached throughout each molecule. Alternatively, nucleic acid scaffolding molecules (components) can be generated synthetically using techniques well known in the art by sequence randomization of individual nucleic acid bases utilizing naturally occurring nucleotides, novel or

unique nucleotides, or nucleotide analogs. (Ecker, D.J. *et al.*, 1993) Oligonucleotide synthesis is a well characterized chemistry that can allow tight control of the composition of the mixtures of oligonucleotides created. Degenerate sequences can be readily produced. Association to linkers or to linker components can also occur after generation of scaffolding components. A library of Morphatides is then created by associating one or more agent molecules, which in one case have also been coupled or connected to other components of the linker molecule or not, with the scaffolding molecules to connect or associate the components of the linker molecules to each other or to connect or associate the agent molecule to the scaffold/linker mini-complex, thereby generating complexes (Morphatides) assembling the scaffolding molecules, linker molecules and the agent molecules. One can use mixtures of agent molecules attached to one type of linker/scaffold attachment site with the present invention. This library is screened to enrich or select for any desired interaction (typically a binding event) with any target (substrate or substrates) of interest. Novel shape and structure libraries, i.e., Morphatides, identified by the methods of screening provided by the present invention are thereby generated. Subsequent to screening, Morphatides may be utilized directly, scaffolding and/or agent molecules and/or complex molecules may be analyzed and mimics of either the entire Morphatide or components thereof may be created for further use, or components of the Morphatides (agent, linker and scaffolding molecules) may be separated, modified, new Morphatides generated and the entire process repeated (iterative selection process). Once a morphatide or collection of morphatides have been identified via the screening process, the structure of one or more of the complexes (morphatides) can be analyzed to retrieve information in order to allow the generation or creation of chemical or small organic molecules that mimic the selected or desired morphatide.

Sequencing techniques can be readily utilized to analyze the scaffolding components of a Morphatide. High-throughput sequencing is well known to the skilled artisan, and the small size of the scaffolding components of Morphatides allows for rapid sequence determination and evaluation of the molecules. It is contemplated herein that other technologies may also be employed to analyze Morphatide molecules. These techniques include utilizing mass spectrometry and nuclear magnetic resonance (NMR) to analyze molecules of interest. Said technologies are well known to the skilled artisan for the evaluation of molecules.

Alternatively, the components of the selected morphatide or collection of morphatides can be separated and the scaffolding component evolved utilizing any of the error prone or sexual PCR or random or directed mutagenesis techniques well known to one of ordinary skill in the art. This process is demonstrated in Figures 2(a) and 2(b) and Figure 3.

Interaction of selected Morphatides and target molecules can occur via binding, contact, connection or other association between the entire complex (Morphatide) and the target, or between any portion of the Morphatide and the target. Each component of the Morphatide can contribute to the shape, structure and/or function of the Morphatide, however actual interaction with a target can occur between any one or more of the components and the target. For instance, any individual component, such as the scaffolding component, linker component, or agent molecule can be the site for binding or associating with the target, or any combination thereof, such as the scaffolding component and the linker component, or the scaffolding component and the agent molecule, etc. can contribute to the binding or association. After desirable Morphatides are identified using the method described, one can further select even more desirable Morphatides by utilizing sexual PCR to effectively eliminate portions of selected Morphatides that do not contribute

to the binding or interaction with the target. Sexual PCR, also known as DNA shuffling, (U.S. Patent No. 5,605,793, entitled "Methods for In-vitro Recombination", Feb. 25, 1997) is employed utilizing modified scaffolding components of the selected Morphatides and similar non-modified scaffolding components to generate further optimized Morphatides. In this process, scaffolding components with attached linkers or linker components of previously identified Morphatides are first separated from the agent molecules and combined with scaffolding components having the same nucleic acid sequence, however not being associated with linkers or linker components. The DNA is then fragmented and reassembled using the sexual PCR technique to generate new scaffolding molecules (components) which are very similar to the original scaffolding molecules, however different. Certain linker attachment sites in the newly generated scaffolding molecules may have been eliminated. Reattachment or association of agent molecules to generate another set of Morphatides can yield a further optimized Morphatide or Morphatides.

In Figures 2(a) and 2(b), an example of the process begins with a template nucleic acid molecule. The template is amplified in the presence of nucleotides which have been previously associated with linker components ("coupling nucleotides"), generating scaffolding molecules. The amplification is performed under conditions to allow random incorporation of the coupling nucleotides. Morphatides are created by, in this example, binding the scaffolding molecules to agent molecules, in this example, chemicals. As used herein, chemicals, include but are not limited to molecules with aliphatic, aromatic, carboxyl, hydroxyl or amine groups. Binding, or desired Morphatides, are selected for by binding to a substrate of interest, and a wash step is used to remove all non-binding substrates and/or Morphatides. In this example, the substrate is then separated from the Morphatide, the chemical group is released. The enriched or selected for

scaffolding molecule(s) are then subjected to amplification under conditions which allow for random incorporation of coupling nucleotides, again, and the process continues as before until the desired Morphatide or collection of Morphatides is finally determined or recovered.

Figure 3 depicts the process in a slightly different format. In this example, the target (substrate) has also been immobilized to a solid surface, and the target (substrate) is separated from the Morphatide after the first screen by elution with a pH shift.

Variable scaffolding molecules can be generated or further modified utilizing a variety of techniques known in the art, including "sloppy", "error-prone" or "mutagenic" PCR, as mentioned. Another technique which may be employed is known as "sexual PCR". Sexual PCR is the forced homologous recombination between nucleic acid molecules of different but highly related sequences in vitro, caused by random fragmentation of the nucleic acid molecules, priming of the fragments on a non-parental nucleic acid molecule based on sequence homology, followed by fixation of the crossover by primer extensions in an amplification reaction. Sexual PCR is used for the in vitro evolution of DNA sequences. The libraries of recombinants that are created by sexual PCR are selected in vitro or in vivo for the best combinations of mutations at the nucleic acid, protein or metabolite level. The process of recombination, selection and amplification can be repeated for as many cycles as necessary to identify the best combinations. Once a collection of Morphatides has been enriched, components of the molecules can be separated from each other, and sexual PCR can be performed to create new scaffolding molecules. After reassociation, the newly generated Morphatides can be further enriched or screened for activity of interest.

Scaffolding molecules can also be generated or further modified by other mutagenic techniques, such as cassette mutagenesis or site directed mutagenesis. Cassette mutagenesis is any process for replacing a small region of a double stranded DNA molecule with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains completely and/or partially randomized native sequence.

It is further contemplated herein that amplification of scaffolding components, or scaffolding components associated with linker components, or Morphatides as a whole, can be achieved in vivo in any aspect of the method of the present invention wherein amplification is beneficial. In vivo amplification can be performed, by example, by utilizing Topoisomerase cloning.

Scaffolding components of the present invention are molecules that contribute to the confirmational diversity and contribute to the complexity of the morphatide libraries to be screened. Diverse scaffolding molecules allow each complex molecule in the library to have a distinct shape. "Shape" has been defined as "the net sum of all the molecular properties and dynamic features that would affect interaction (of molecules) with other molecules". (Kenan,D.J. *et al.* 1994) Nucleic acid scaffolding components of the present invention can also provide the capability to clone, amplify, and evolve components of the morphatides and to decode the structure of the selected morphatides.

Nucleic acid scaffolding molecules can consist of single stranded, double stranded, triple stranded or branched DNA or RNA molecules. DNA or RNA scaffolding molecules can be generated using synthetic or biosynthetic methods. Nucleotide analogs to nucleotide bases, such as 5-azo-cytodine, inosine or 7-deazaguanine can be employed to increase the complexity of the resulting scaffolding molecules. Nucleotide bases may be modified prior to, or after, generation of

nucleic acid scaffolding molecules. Nucleotide scaffolding molecules can be generated by randomization of the order of individual bases or modified bases. (Houghten, 1985; Beaudry and Joyce, 1992) Such techniques are well known to those skilled in the art.

Scaffolding molecules of the present invention can comprise a variable core flanked by short sequences to facilitate amplification. The variable core can be designed such that the sites of connection to the linker can be readily identified. This could facilitate the rapid determination of the structure of an optimized scaffolding molecule component of a Morphatide. For example, by constructing the variable core as follows, this challenge can be resolved: since a polynucleotide comprising just 3 of the 4 normal bases provides adequate variation for the scaffolding molecule, the remaining base can be used to serve as the connector or attachment site for the linker to carry the agent molecule. By having one base function as the attachment site for the linker in the construction of the variable core, the number of linker sites can be controlled and the position readily ascertained.

Alternatively, any one or more of the different bases or any base mimic can be used as an attachment site. Preferably, sites of connection of the linker and agent are internal in the scaffolding component to potentially provide greater shape and structural diversity. Connection sites restricted to terminal sites in the scaffolding components may limit this desired feature. It should be noted that if one were only utilizing a modified nucleotide to interact or bind to a target of interest, internal connection or modified sites could severely interfere with any further hybridization event of this nucleotide. With the present invention, however, the greatest shape and structural diversity can

be provided by utilizing internal sites of the scaffolding molecules for connection.

It is recognized that novel, or unique bases or base analogs, not included in the 4 normal bases, can be employed in the present invention. Such novel or unique bases or base analogs can include, but are not limited to, bases, base analogs or "mimics" such as difluorotoluene deoxynucleosides,(Rawls, R., 1997) or other molecules which can be incorporated by any nucleic acid polymerase to any degree, and/or which do or do not effect hybridization, binding or association of a nucleotide to any other molecule or molecules.

It is also recognized that more than one nucleic acid polymerase can be employed simultaneously to incorporate bases or base analogs and/or "linker" attached bases or base analogs.

Scaffolding molecules can also be modified post generation via a variety of chemical techniques, such as that employed in the Maxam and Gilbert DNA sequencing procedure, or via the utilization of known mutagens such as UV light, or DNA binding proteins which can cause modifications to specific or random nucleotides. The Maxam and Gilbert DNA sequencing procedure is very well known in the art. (Maxam, A.M. and Gilbert, W. 1980) The procedure involves the treatment of DNA samples with a chemical that specifically damages or modifies one or two of the four bases in DNA in a controlled reaction where only a few of the sites are nicked in any one DNA molecule. The chemicals utilized in this procedure can be utilized in the present invention. Mutatable nucleotides, such as methylated cytosines may be utilized to generate scaffolding molecules. Mutagens such as (+)-CC-1065; (+)-CC-1065 (N3-Adenine); trivalent chromium or trivalent chromium salts; polycyclic aromatic hydro-carbons (PAH), such as 7-bromomethyl-benz[a]anthracene (BMA); Tris(2,3dibromo-propyl)phosphate (Tris-BP);

1,2-dibromo-3-chloro-propane ("DBCP"); 2-bromoacrolein (2BA); benzo[a]pyrene-7,8-dihydrodiol9-10-3epoxide (BPDE); platinum(II) halogen salts; N-hydroxy-2amino-3-methyl-imidazo[4,5-f]-quinoline (N-hydroxy-]Q); or N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-f]-pyridine (N-hydroxy-Ph]P) can be added to modify nucleic acids by adding chemical adducts to specific or random nucleotides. These adducts or adducted sites may then be utilized as linkers to which agent molecules, hereinafter defined, can be attached, or can themselves act as agent molecules.

Agent molecules of the present invention are molecules that further provide for complex and diverse morphatides. As used herein, an agent molecule is a molecule that can be recognized by or can recognize a particular target molecule or portion of a target molecule of interest. This recognition is typically a binding event or some other event of association. Screening of and selection from shape and structure libraries (Morphatides) represents an approach to generating complexes of molecules that recognize and bind target molecules. The molecules that recognize and bind target molecules may be the entire complex of molecules (the Morphatides) or individual components thereof, including but not limited to the agent molecules or the scaffolding components.

Agent molecules can consist of natural products such as natural polymers (peptides, oligonucleotides, etc.) or natural nonpolymeric molecules (antibodies, etc.), or of unnatural products such as synthetic polymers or other synthesized nonpolymeric molecules. Examples of agent molecules include, but are not limited to, peptides, nucleic acids, carbohydrates, proteins, and other molecules synthetic compounds, agonists and antagonists for cell receptors, hormones, chemicals, chemical structures, sugars, cofactors, enzymes and other proteins, enzyme substrates, and drugs. There are virtually an unlimited number of agent molecules

that may be screened using the present invention. Assembly of agent molecules can occur by systematic association of building block components of agent molecules using chemical, biological, or biosynthetic procedures.

Where the agent molecule is a nucleic acid, it is possible to access even more diverse shapes. In this particular embodiment, as both the agent and scaffold are nucleic acid based, either the agent, the scaffold or both can be evolved according to the methods described herein. Thus, one can easily analyze (deconvolute) the molecule from either the scaffold or agent. The potential shapes that can be created increase dramatically, since the new agent can achieve many different shapes. This concept of utilizing a nucleic acid as an agent molecule in a Morphatide represents a "combination, combinatorial" approach.

The use of one or more nucleic acids as agent molecules allows one to create associations to multiple sites of the agent molecule and/or scaffold component. Thus, one can generate the scaffold with one portion of the linker component, the agent with the other portion and combine the two to yield a new molecule. Linker components can be added to any desired position or positions on either the agent or scaffold, thus increasing the diversity of the resulting population. In addition, a single agent can be linked to the scaffold at more than one position through the use of multiple linkers. One linker type can be used to associate the agents to scaffolds, or more than one linker type can be used to associate one or more agent molecules to one or more scaffold molecules. Agents can be associated to scaffolds in a one-to-one ratio, whereby an agent molecule is associated with the scaffold at one position, or agents can be associated to scaffolds in any ratio in excess of one to one, whereby more than one agent molecule is associated with the scaffold at various positions. Where multiple agents are used, the agent may comprise the same or substantially

the same sequence at each position on the scaffold, or may comprise diverse sequences. It will be understood that nucleic acid agents may be used with any combination of other types of agent molecules described above. Agent molecules can be "stacked" upon one another, to yield complex molecules, whereby multiple agent molecules associated with each other are associated to scaffolding components. Thus, the use of a nucleic acid as an agent can allow one to create a variety of associations between agent and scaffold components.

Nucleic acid scaffold and/or agent components can fold upon themselves creating "internal" or "intra-strand" associations, providing even further shape diversity, especially when combined with "inter-strand" associations.

Agent, linker and scaffold components can be separated after enrichment for one or more desirable characteristics, and either agent or scaffold or both components can be evolved using any method described to yield new, potentially more-desirable components, which can be re-associated to be screened. Alternatively, desirable molecules can be utilized directly.

The terms "agent" and "scaffold" have been used in describing this particular embodiment to distinguish two or more nucleic acid components. However, it is recognized that where both components are nucleic acids, an "agent" could also be considered a "scaffold".

Synthesis methods are well known in the art. Building block components of agent molecules can be diverse and fairly complex. Assembly of such building blocks could yield a broad, diverse collection of agent molecules providing diverse physicochemical properties, functionality, charge, and conformation. Building blocks could have groups with high reactive functionality that

allow for multiple new covalent combinations and many potential connecting permutations providing diverse, spatial relationships (carbohydrates, for example, where almost every carbon in a given molecule has a hydroxyl or other oxygen-containing functional group attached to it).

Linker molecules of the present invention are molecules that can allow connection or association of the scaffolding molecules to agent molecules. Linker molecules can consist of chemical compounds. Typically, the chemical compounds contain one or more reactive groups, allowing the linkers to associate and preferentially to be cleaved or disconnected by means of a specific reaction or reaction steps. Linkers also have appropriate functional groups at each end for coupling to the scaffolding molecules and to the agent molecules. Preferred linkers are those whose cleavage or disconnection is controllable. Particularly preferred linkers are those whose cleavage or disconnection is reversible. Illustrative examples of suitable linkers include bioconjugates such as Phenylboronic Acid, DNA binding proteins, or biotin/streptavidin. In addition to the cleavable groups, suitable linkers may contain other groups that influence or do not influence the cleavage reaction, which are suitable for enriching or separating scaffolding molecules from agent molecules.

Cleavable linkers may also consist of a cleavable component and a constant component, which is the same for either all scaffolding molecules or for all agent molecules. The constant part may consist of chemical compounds which permit attachment to both the cleavable part of the linker and to other chemical groups or to other molecules. An example of a constant component is an invariable part of the scaffolding molecule or of the agent molecules.

Nucleotide molecules can be used alone as scaffolding molecules, or linker molecules can be employed in the present invention to force

the shape of the resulting complex molecules to yield novel shapes, or scaffolding molecules.

There are a variety of linkers that may be useful for purposes of the present invention. For instance, linker molecules could be based upon the Phenylboronic acid complexing moieties (Yurkevich 1969). Phenylboronic acids are known to interact with a wide range of polar molecules having the requisite functionalities (Middle 1983; Frantzen 1995). Phenylboronic acid, like boric acid, is a Lewis acid, and ionizes not by direct deprotonation, but by hydration to yield the tetrahedral phenylboronate anion (pKa=8.86). A variety of phenylboronic acid molecules with varying pKa's are commercially available. Molecular variations can also be generated. Ionization is fundamental for complexation causing a change from trigonal coordination to tetrahedral coordination. Bioconjugation with phenylboronic acid molecules has been achieved between compounds having diol functionalities (e.g. carbohydrates) to immobilized phenylboronate anion to form cyclic esters under alkaline conditions. Release is effected by pH shifts. Phenylboronic acid modified dUTP linker molecules have also been incorporated into oligomers using DNA polymerases as an alternative to DNA labeling and purification via biotin incorporation. Bioconjugation via linkers such as the phenylboronic acid linker can simplify the reversibility of the coupling reaction, enabling attachment of agent groups that cannot generally be incorporated by DNA polymerases. In addition, the phenylboronic acid molecule causes minimal interference with respect to DNA hybridization and base incorporation with a deoxynucleotide triphosphate attached to it.

Phenylboronic acid bioconjugate complexes are suitable for use as linker molecules in the present invention. It is a preferred embodiment. Methods for associating and dissociating suitable linkers to many different types of potential molecules, such as the

agent molecules and/or scaffolding molecules referred to in the present invention are known to one of ordinary skill in the art. (WO 95/20591) These methods include but are not limited to those described in WO 95/20591, as well as those using biotin-streptavidin. Figure 4 depicts the use of this type of candidate bioconjugate, indicating the fact that standard chemistry can be used to attach one component of the bioconjugate (linker) to candidate scaffolding molecules (Binder 1 or 2), and to attach another component of the linker to candidate agent molecules (Binder 2 or 1, depending on which Binder scaffolding molecule is). A condensation reaction then associates the linker components, creating a complex, or Morphatide.

Disulfide based coupling systems can also be utilized as linkers in the method of the present invention. Disulfide based coupling systems, such as that described herein, offer the benefit of being reversible under mild redox conditions. The system is selective for thiol groups and nucleic acid is stable under the conditions in which the system functions.

In the system, depicted below, the nucleoside triphosphate can be linked by an amine containing linker arm attached to the nucleoside base component of a scaffolding molecule. An agent molecule can then be linked by a thiol containing linker arm.

Heterobifunctional cross-linkers, such as N-succinimidyl 3-[pyridyldithio]propionate (SPDP) (described by Hermanson, Greg T., Bioconjugate Techniques, Academic, San Diego, CA 1995, p.230)(available from Pierce), which link amino containing molecules to a thiol containing molecule can be employed. The heterobifunctional cross-linker SPDP is available from Pierce as are other related crosslinkers with different chain lengths making this a versatile system for manipulation of the distance between the DNA oligomer and the chemical group of interest.

The 5-position of cytidine and uridine, and the 8-position of adenine and guanine are preferable positions for association with the linker. It is contemplated that other positions can be utilized.

Although other potential systems may be used, they lack the reversibility, ease of manipulation and relative ease of synthesis that the disulfide system possesses. For example, the diazirine coupling system could be used to incorporate the linker into a modified nucleotide containing a thiol group through the maleimide portion and the chemical group (CG) through the aryldiaziridine portion. However, the system is less selective and less reproducible than the disulfide coupling system.

It is contemplated that modified nucleotide molecules, such as pseudouridine or other molecules which can be utilized to generate polymers similar to nucleic acid, but which are more stable than nucleic acids, such as PNA monomers, can also be utilized with the methods of the present invention.

Nucleotides associated with linker molecules are incorporated into scaffolding components. Morphatides are generated on treatment of the scaffolding components with agent molecules derivatized to contain thiols.

As used herein a binder is any molecule which is to be attached to another molecule by the linker. Binders include but are not limited to nucleic acids, amino acids and chemical groups. Therefore, the components of a Morphatide, i.e., the scaffolding component and the agent molecules are binders.

Linkers can be coupled via functional groups to one or more different sites on both scaffold molecules and agent molecules. Synthesis methods to attach phenylboronic acid to other molecules

are known (WO 95/20591). Connecting scaffold molecules to agent molecules via a reversibly connectable linker such as phenylboronic acid yields conformationally diverse library complex molecules.

Target molecules of the present invention are molecules to which morphatides are selected to bind, associate, or interact. Target molecules can be any molecule of interest. Examples of such molecules include, but are not limited to, cell membrane receptors, antibodies, lectins, polysaccharides, cells, cellular membranes, organelles, and chemicals. Preferably, target molecules of interest are bound to a support. Examples of such supports include, but are not limited to, solid surfaces, beads, particles, or other support.

Libraries of complexes (morphatides) can be screened for any target molecule of interest, any chemical activity such as catalysis of inorganic and organic reactions, or any biological activity of interest which may be known in the art. Biological activities known in the art include, but are not limited to, antimicrobial activities, antitumor activities, enzyme inhibiting activities, receptor binding activities, growth promotion activities, antibody binding activities and biofilms. Many screening assays are available and known for these activities and a variety of other biological responses, and any can be used with the present invention.

Certain groups have disclosed the use of miniaturization, or chip technology to allow one to screen molecules for activities of interest. For example, U.S. Patent numbers 5482867, 5445934 and 5605662 describe strategies to allow one to carry out desirable reactions in micro formats. Such screening technologies may be used to analyze Morphatides to identify characteristics of interest, such as the ability of a Morphatide to bind to a target.

Evolution via such techniques as previously described, such as Sexual PCR, may be performed on undesirable scaffolding molecules subsequent to identification and separation from desirable complexes to eventually create new collections of complexes which can be rescreened for desirable activity. Undesirable scaffolding molecules are those scaffolding molecules that are part of the Morphatides that do not bind or pass the screening test.

A variety of screening techniques are known in the art and can be used in the present invention. (Mullinax, R., et. al., 1990; Barbas, C., et. al., 1991; Castagnoli, L., et. al., 1991; Garrard, L., et. al., 1991; McCafferty, J., et. al., 1990; Clackson, T., et. al., 1991; Kang, A., et. al., 1991; Hoogenboom, H., et. al., 1991; Chang, C., et. al., 1991;)

It is recognized that with any screening technique involving a binding event, steps may be taken to decrease potential nonspecific binding of Morphatides. These steps are well known in the art, and include but are not limited to enriching, isolating or separating bound or otherwise associated complexes from unbound or unassociated complexes can occur via a variety of enrichment, isolation and separation techniques well known in the art. Typical enrichment, isolation and separation techniques involve solvent partitioning and/or conventional chromatography.

Enriching, isolating or separating bound complexes (morphatides) from target molecules can occur via a variety of isolation and separation techniques, also well known in the art, and depend on the nature of the connection between the complex and the target molecule. Typical enrichment, isolation and separation strategies involve elution or digestion steps.

More particularly, the present invention provides a method for identifying one or more complexes from a library of complexes,

wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; (ii) one or more linker components; and (iii) one or more agent molecules or type of agent molecules, linked to the scaffolding component by one or more type of linker components; and (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure; (c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure.

Another aspect of the present invention provides a method for identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding component selected

from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; and (ii) one or more agent molecules or type of agent molecules, associated, bound, or bonded to the scaffolding component; (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure; (c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure.

The contacting, binding, or associating of the morphatides to target molecule(s) or of a morphatide conjugated to a therapeutic agent(s), as described infra, may be through ionic, covalent, hydrophobic or hydrogen bonds, or through Van der Waals forces.

In an embodiment of the above-described methods, the separation of step (c) is performed by either (a) separating the morphatides which do not perform the preselected or desired function or which do not bind or associate through a pre-selected structure or (b) separating the morphatides which perform the preselected or desired function or which bind or associate through a preselected structure.

Methods of separating are well known to one of ordinary skill in the art and include but are not limited to centrifugation; electrophoresis; biopanning; solubility differences; chromatography; fluorescence sorting, properties such as physical, chemical or electrical; photochemical; magnetic; and visible detection.

In a preferred embodiment of the above-described methods said target molecule is bound to a solid support. In a preferred embodiment the preselected or desired function performed by the complex(es) on a target molecule is selected from the group consisting of binding to or associating with said target molecule; reacting with said target molecule and changing the property of said target molecule; having an affinity for and binding to a specific ligand; performance of a biological activity, wherein said biological activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

In another embodiment the contacting, binding or association is selected from the group consisting of multiple complexes acting on a single target molecule, a single complex acting on multiple target molecules, components of one or more complexes acting on multiple target molecules, components of one or more complexes acting on a single target molecule, and multiple complexes acting on multiple target molecules.

In a preferred embodiment the scaffolding component comprises naturally occurring nucleotides, novel or unique bases, base analogs or any combination thereof. In an embodiment said

scaffolding component comprises synthetically generated nucleic acids, nucleic acid like molecules, nucleic acid analogs. In an embodiment the nucleic acid like molecule is a difluorotoluene or related deoxynucleoside.

In another embodiment of the above-described methods said scaffolding component consists of subunits which are capable of being incorporated by one or more nucleic acid polymerases or reverse transcriptases and which can, when polymerized, generate hybridizable polymers with hydrogen bonding or hybridizable polymers without hydrogen bonding. In another embodiment said scaffolding component comprises nucleic acids having regions of conserved sequences and one or more regions of randomized sequences. In a preferred embodiment the scaffolding component(s) are comprised of two fixed regions of nucleotides and one region of randomized nucleotides between the two fixed regions. In an embodiment the linker component is associated to a base of the scaffolding component either before or after the scaffolding component is made. In another embodiment the randomized region is comprised of: (a) three of the four bases occurring with similar frequency; and (b) one of the four bases occurring at a rare frequency. In an embodiment one of the bases occurring with similar frequency is associated with or binds with the linker component. In an embodiment one of the four bases occurring at a rare frequency is associated with or binds with the linker component. In an embodiment the position of the base with the linker attached is determined by nucleotide sequencing or mass spectrophotometry.

In another embodiment each scaffolding component comprises more than one different nucleic acid base being attached to a linker component, said nucleic acid base being incorporated into the scaffolding component either during PCR amplification or during synthesis of the nucleic acids. In an embodiment the incorporated nucleic acid base to which the linker component is attached is a

rare base. In an embodiment the base to which the linker is attached is modified, said modification being by chemical reaction either before or after incorporation during PCR amplification or during synthesis of the nucleic acids.

In further embodiment the incorporated nucleic acid base to which the linker component is attached is a different modified base, said base being any of the four bases or analogs of said bases, said base being modified by a reaction. In a preferred embodiment the incorporated base to which the linker component is attached is located internally in the scaffolding component.

In a further preferred embodiment one or more of said linker components are reversible. In an embodiment one or more of said linker components are non-reversible. In an embodiment one or more of said linker components cannot be amplified (any kind of mathematical increase in the number of molecules) in vitro or in vivo. In another embodiment one or more of said scaffolding components associated with one or more of said linker components is amplifiable in vitro or in vivo. In an embodiment said one or more of said linker components associated with one or more of said agent molecules cannot be amplified in vitro or in vivo. In a preferred embodiment the entire morphatide is amplifiable. In a further preferred embodiment one or more of said linker components comprise reversibly connectable components or parts.

In an embodiment a first linker component either reversibly or non-reversibly associated with a scaffolding component and a second linker component either reversibly or non-reversibly associated with an agent molecule are connected together to generate a scaffolding component linked to an agent molecule by the connectable first and second components of said linker component. In a preferred embodiment the linker component is selected from the group consisting of a phenyl-boronic acid linker, a thio linker,

and a biotin-streptavidin linker. In an embodiment the thio linker is cysteine. In another embodiment the scaffolding component is associated to one or more agent molecules, wherein said agent molecule is a peptide by a peptide bond. In an embodiment the linker component is selected from the group consisting of a nucleic acid binding protein and a chelating molecule. In an embodiment the linker component is bound covalently to either the scaffolding component or to the agent molecule. In another embodiment the linker component is bound noncovalently to either the scaffolding component or to the agent molecule. In an embodiment of the above-described methods said agent molecules are selected from the group consisting of naturally occurring polymers, synthetically generated polymers, and non-polymeric molecules.

In another embodiment of the above-described methods the library of complexes is prepared by: (a) coupling the linker molecules or components of the linker molecules to either the scaffolding components, to form scaffolding component-linker molecules or to the agent molecules, to form agent molecule-linker molecules; and (b) generating a linkage between the scaffolding component-linker molecules and the agent molecules or between the scaffolding components and the agent molecule-linker molecules to yield the complexes, thereby preparing a library of complexes. In an embodiment said scaffolding components are prepared for coupling to linker molecules via chemical reaction yielding modified nucleotides. In a further embodiment said chemical reaction involves treating the scaffolding components with one or more mutagens to add one or more base specific or non-specific adduct(s), resulting in adducted scaffolding molecules, that enable increased reactivity of the base to the linker or directly to the agent molecules. In a still further embodiment said chemical reaction involves treating the scaffolding components with one or more mutagens to add one or more base specific or non-specific adduct(s), resulting in adducted scaffolding molecules, said adduct

acting as either a linker or an agent molecule. In another embodiment the adducted scaffolding components are amplifiable. In a preferred embodiment said mutagen is UV light, any other nucleic acid mutagen, or a nucleic acid binding protein. In a further preferred embodiment said chemical reaction involves treating scaffolding components with Maxam & Gilbert based chemistries to generate increased reactivity of one or more bases to a linker or to an agent molecules.

In another embodiment said scaffolding components are prepared for coupling to the linker molecules via a non-chemical reaction yielding modified nucleotides. In an embodiment said non-chemical reaction is an enzymatic reaction.

In further embodiment of the above-described methods said methods further comprise after step (b): (a) disassociating the scaffolding component of the complex performing the preselected or desired function from the agent molecule or molecules; (b) generating modified scaffolding components; (c) associating the different scaffolding molecules with agent molecules to generate different morphatides; (d) rescreening the different morphatides by repeating steps (b) and (c) of claims 1 or 2 to identify new desired candidate morphatides.

In an embodiment said modification of the scaffolding components occurs via a random or directed mutagenesis technique. In an embodiment said random or directed mutagenesis techniques are selected from the group consisting of error-prone PCR or sexual PCR by performing a suitable number of cycles on the scaffolding components, resulting in one or more base changes in some percentage of the scaffolding components; cassette mutagenesis; and site directed mutagenesis. Such techniques are well known to one of ordinary skill in the art. One example of a random mutagenesis technique is termed "PCR mutagenesis" (PCR Primer, A Laboratory

Manual, Cold Spring Harbor Laboratory Press 1995). Another mutagenesis technique, previously mentioned, is termed DNA shuffling or Sexual PCR (W. P. C. Stemmer, 1994). Another mutagenesis technique known to one of ordinary skill in the art is Combinatorial Multiple Cassette Mutagenesis [Biotechniques, (1995)]. In vitro evolution procedures of nucleic acids have also been described by several other groups over the last many years (A. Beaudry and G. Joyce).

Another technique, hereinafter referred to as breeding, may be used. Breeding is similar to the process of shuffling without the fragmentation step. Shuffling, as known in the art, begins with a collection of similar sequences (~80% homology) typically genes encoding molecules of interest. The sequences are treated with DNase to fragment the nucleic acid, and amplification takes place with the fragments. The nucleic acid sequences in a Morphatide are relatively short fragments (hundreds of bases), compared to the size of most genes (thousand(s) of bases). With breeding, evolution of a Morphatide, or collection of Morphatides, can take place without the fragmentation step normally associated with the shuffling process. Crossover amplification (PCR) of the sequences of the scaffolding components may be performed to achieve breeding of the molecules. The example provided herein details breeding fragments, utilizing a hybridization/extension/ amplification reaction (a process similar to cross-over PCR).

Scaffolding component(s) from a Morphatide or collection of Morphatides can also be modified in vivo utilizing host organisms deficient in mismatch repair systems.

One can rely on homologous recombination between areas of homology present in the scaffolding component of the Morphatides in an enriched or unenriched collection. Homologous recombination can be employed in an in vivo strategy of breeding molecules. The term

"homologous" means that one single-stranded nucleic acid sequence may hybridize to a complementary single-stranded nucleic acid sequence. The degree of hybridization may depend on a number of factors including the amount of identity between the sequences and the hybridization conditions such as temperature and salt concentration. Preferably the region of identity is greater than about 5 base pairs. Homologous recombination is well known to the skilled artisan.

Scaffolding components from selected or unselected Morphatides are rendered double stranded, ligated into a vector, and transformed into a host organism, utilizing techniques well known in the art. Sequences of homology present in the sequences of the scaffolding components result in recombination of the sequences, generating new molecules which can be recovered using techniques known to the skilled artisan. The recovered scaffolding components can be utilized to generate new Morphatide molecules and screened for an activity of interest utilizing techniques described herein.

Any vector which will propagate in a host organism of choice may be utilized. Vectors allowing multiple scaffolding components to be cloned into a single vector are preferable. For example, "polycos" vectors (Alting-Mees M.A. and Short J.M., Gene, 1993 Dec 27, 137:1, 93-100) can be utilized. Any other vector, including M13 and phagemid cloning vectors, can also be utilized, and final constructs can be co-transfected into the host organism of choice. Systems for cloning PCR fragments can be utilized. For example, the TA Cloning system available from Invitrogen Corporation in Carlsbad, CA, or the PCR-Script Cloning system available from Stratagene Cloning Systems may be employed. Multiple types of vectors can also be utilized as long as their origins of replication are compatible in the host. Cloning vehicles containing scaffolding components are used to transform a host cell. Such host cell preferably is deficient in mismatch repair systems. Said host

cells are well known to the skilled artisan. For example, XL1-Red cells are mismatch repair deficient and are commercially available (Stratagene Cloning Systems, La Jolla, CA).

It is contemplated that the above described process of homologous recombination could be performed in vitro, if one were to include in the reaction necessary reaction components to allow homologous recombination between two or more nucleic acid strands.

Once two or more Morphatides of interest have been identified, scaffolding component sequences may be analyzed, for instance by sequencing the scaffolding components, and utilized to generate smaller sequences, such as 30mer sequences, which represent regions of the scaffold sequence. These smaller sequences may be generated synthetically or biologically utilizing techniques well known in the art. Said smaller sequences may be employed in a shuffling reaction to generate novel scaffolding molecules. Hence, rather than fragmenting the sequences as is performed in standard shuffling, known oligonucleotide sequences are generated and "bred." It is contemplated that oligonucleotide sequences can also be employed to generate new longer sequences utilizing ligase to build longer molecules, versus polymerase to extend in a crossover amplification reaction.

It is contemplated that evolution of Morphatide molecules can occur between scaffolding components of Morphatides, scaffolding components associated with linker moieties, or Morphatide molecules as a whole. Where scaffolding components or scaffolding components associated with linker moieties are bred, linkers and/or agent molecules can be associated subsequent to the reaction. Morphatide molecules which can be evolved as a whole do not need to be dissociated into smaller components prior to the evolution process.

Once Morphatides have been evolved, and enriched or selected for optimal candidate molecules, a process of "deconvolution" may be performed to find even more optimal molecules. The evolution process of the method of the present invention allows one to significantly increase the complexity (diversity) of the sample to be screened. The best molecules for the desired activity are selected from the most diverse sample possible. Subsequent to evolution and screening, the complexity of the newly identified Morphatides may be decreased to find an even further optimal activity of interest. "Deconvolution" is a process whereby any non-essential positions of linkers/agent molecules associated with the scaffolding components can be eliminated. The process of deconvolution allows one to select against these non-essential positions. For example, molecule (A) may be determined to possess a desirable activity.

However, molecule (B) may possess optimal activity, but may not have been present in the original diverse sample. The process of "deconvolution" allows one to identify (B) from a new collection of Morphatides consisting of every combination of linker/agent association sites represented in a Morphatide. By way of example, if Morphatide (A) has 5 sites of association of linker/agent molecules. The new collection generated via the process of deconvolution would have molecules containing only the first, second, third, fourth, or fifth sites, the first and second, first and third, first and fourth, or first and fifth sites, the second and third, second and fourth, second and fifth sites, etc. Thus from this collection, one can identify an optimal molecule.

The process of deconvolution can be performed by first generating oligonucleotides representing sequence of the scaffolding components of identified Morphatides. "Linker" bases or combinations of linker bases would be represented on different oligonucleotides sequences. New molecules can then be built from

these shorter sequences using crossover amplification strategies or ligase strategies described above.

In another embodiment one or more of said agent molecules in step (c) are different from the agent molecules utilized in the morphatides of the prior round of screening for identification of morphatides performing the preselected or desired function.

In another embodiment of the above-described methods identifying a different morphatide further comprising: (a) separating the scaffolding components from the agent molecules; (b) performing a suitable number of cycles of error prone PCR on the scaffolding components, resulting in one or more base changes in some percentage of the scaffolding components; (c) reconnecting the scaffold component to the agent component; and (d) repeating steps (a) through (d) of the above-described methods, thereby identifying a different morphatide. In an embodiment the morphatide comprises a linker component, wherein in step (a) one part of a linker remains attached to the scaffold component and another part of the linker remains attached to the agent molecule. In another embodiment in step (c) both parts of the linker are connected, thereby reconnecting the scaffold component to the agent component. In a further embodiment the connection between the agent molecule and the scaffolding component is by a plurality of the linker component, i.e., pieces of the linker component. In an embodiment the scaffolding components are characterized by cloning and nucleotide sequencing before reattachment of the agent molecule in step (c).

A further embodiment of the above-described methods comprises (a) creating a mimic of the identified morphatide; and (b) using the mimic for a desired application.

In a still further embodiment the above-described methods further comprise: (a) separating scaffolding components with attached linker components or parts of the linker components from the agent molecules of the previously identified Morphatides; (b) combining the scaffolding components with attached linker components or parts thereof with scaffolding components comprising a same nucleic acid sequence as the scaffolding components, said nucleic acid sequence not being attached to or associated with a linker components or parts thereof, thereby resulting in nucleic acid sequences without the one or more linker sites; (c) using sexual PCR to fragment and reassemble the nucleic acid sequences, resulting in elimination of linker component sites which do not contribute to the binding of the morphatide, thereby generating new scaffolding components similar but not identical to the scaffolding components of step (a); (d) reattaching or association agent molecules to the new scaffolding components of step (c), thereby generating another set of Morphatides.

Yet another aspect of the present invention provides a method of identifying a presence of a substance in a sample from a subject, comprising: (a) obtaining a sample; (b) contacting the sample with one or more types of morphatide identified by either of the methods for identifying one or more complexes so as to form a complex between the morphatide and the substance present in the sample; (c) detecting the complex formed in step (b), thereby identifying the presence of the substance in the sample from the subject. In an embodiment step (c) is performed by PCR amplification, ethidium bromide staining or labeling selected from the group consisting of radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and a ligand. In a preferred embodiment the detection of the complex formed in step (c) comprises identification of an occurrence selected from the group consisting of binding to or associating with a target molecule; having an affinity for and binding to a specific ligand; performance of a

biological activity, wherein said biological activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

This invention further provides a method of diagnosing a subject wherein detection of a complex in step (c) of a method of identifying a presence of a substance in a sample is indicative of a disease. In an embodiment the sample is a body fluid or a tissue specimen. In another embodiment the body fluid is selected from the group consisting of blood, serum, plasma, urine, saliva, nasal mucosal discharge, vaginal mucosal discharge, anal mucosal discharge, peritoneal fluid, cerebrospinal fluid, and lymphatic fluid. In a further embodiment the substance whose presence is identified is selected from the group consisting of hormones, enzymes, proteins, cancer/tumor cells, pathogens, and drugs. In a still further embodiment the subject is bacterial cells, a plant, a microbe, an insect, a fish, or a mammal. In a preferred further embodiment the mammal is a human.

Still another aspect of the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with multiple types of molecules. Another aspect of the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with one type of molecule.

In a preferred embodiment of the morphatide the binding to, crosslinking with, or reacting with the molecules is selected from the group consisting of binding to or associating with a target molecule; having an affinity for and binding to a specific ligand; performance of a biological activity, wherein said biological

activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

This invention provides a composition comprising the preferred embodiment of the morphatide effective to treat a subject and a pharmaceutically acceptable carrier.

This invention also provides a method of administering the above-described compositions, wherein the administration is intravenous, intraperitoneal, intra-thecal, intralymphatical, intramuscular, intralesional, parenteral, epidural, subcutaneous; by infusion, liposome-mediated delivery, aerosol delivery; topical, oral, nasal, anal, ocular or otic delivery. In a preferred embodiment the morphatide is conjugated to a therapeutic agent. In an embodiment the therapeutic agent is a radioisotope, toxin, toxoid, or chemotherapeutic agent.

This invention further provides a composition comprising the above-described conjugated morphatide and a pharmaceutically acceptable carrier, wherein the morphatide is selected from either a morphatide which is capable of being degraded or a morphatide which is incapable of being degraded after administration to a subject.

The present invention also provides a pharmaceutical composition comprising a effective amount of the morphatides described above and a pharmaceutically acceptable carrier. In the subject invention an "effective amount" is any amount of an morphatide which, when administered to a subject suffering from a disease or abnormality against which the morphatides are effective, causes reduction,

remission, or regression of the disease or abnormality. In the practice of this invention the "pharmaceutically acceptable carrier" is any physiological carrier known to those of ordinary skill in the art useful in formulating pharmaceutical compositions.

In one preferred embodiment the pharmaceutical carrier may be a liquid and the pharmaceutical composition would be in the form of a solution. In another equally preferred embodiment, the pharmaceutically acceptable carrier is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical carrier is a gel and the composition is in the form of a suppository or cream. In a further embodiment the compound may be formulated as a part of a pharmaceutically acceptable transdermal patch.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The

liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compounds may be prepared as a sterile solid composition which may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Carriers are intended to include necessary and inert binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings. The compositions may be further comprised of stabilizers, which as used herein, is a substance which increases the half-life of the morphatide in the blood stream. Polyethylene glycol may be used as a stabilizer. A stabilizer would comprise the composition, for example when the morphatide of the composition is to be degraded after administration so as to deliver a bound therapeutic agent which is to be time released.

The morphatides can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents, for example, enough saline or glucose to make the solution isotonic, bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like.

The morphatides can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular morphatide in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition or abnormality. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

The present invention provides a morphatide labelled with a detectable marker. In an embodiment the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and a ligand.

In an embodiment of the above-described compositions wherein the morphatide is incapable of being degraded the composition further comprises a stabilizer molecule for increasing the half-life of the morphatide in the blood stream. In an embodiment the stabilizer is polyethyleneglycol.

This invention provides a method of treating a subject with any of the above-described compositions of morphatides and morphatides conjugated to therapeutic agents.

In another aspect, this invention provides a method of drug delivery to a target in the body of a subject comprising administration to a subject any of the above-described compositions of morphatides and morphatides conjugated to therapeutic agents, thereby delivering the drug to the target.

In an embodiment of the above-described compositions wherein a morphatide is capable of being degraded, the degradation is performed by either of a nuclease or protease.

This invention further provides a method of drug delivery to a target in the body of a subject comprising administration to a subject of the above-described compositions of morphatides and morphatides conjugated to therapeutic agents, wherein the morphatide is incapable of being degraded or is slowly degraded after administration to the subject, thereby delivering the morphatide-bound drug to the target. In an embodiment of the method of drug delivery, wherein the morphatide-bound drug is administered, the administration may be intravenous, intraperitoneal, intrathecal, intralympha-tical, intramuscular, intralesional, parenteral, epidu-ral, subcutaneous; by infusion, liposome-mediated delivery, aerosol delivery; topical, oral, nasal, anal, ocular or otic delivery.

In a further embodiment of the above described methods of identifying morphatides, the morphatide is capable of binding to any component of an antibody molecule, said antibody having a constant and variable region.

It is contemplated that products of the present invention are useful in dideoxy-sequencing, cycle-sequencing, restriction enzyme based and other similar sequencing strategies, the methods of which are well known in the art.

In standard dideoxy sequencing reactions, template nucleic acid molecules are incubated in four separate reactions with primers, nucleotides (all four), and dideoxy (chain terminator) nucleotides (different dideoxy nucleotide for each reaction). After primer hybridization, polymerase is added to the mixture and extension proceeds. The incorporation of a dideoxy nucleotide terminates the extension reaction. The presence in a reaction of both regular nucleotide and the terminator version of a nucleotide ensures that the termination event will not occur every time the nucleotide is required to be incorporated. Thus, this random incorporation of dideoxy nucleotides yields fragments, which can be distinguished based on size when denatured and separated by electrophoresis. The procedure is described in detail in Sambrook, Fritsch, Maniatis, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989.

Fragments generated by the above method are labeled for detection by a variety of methods. Radioactive, or fluorescent or infrared dye labels are employed. Primers are labeled, or chain terminating molecules (for example, dideoxy nucleotides) are labeled. One problem encountered with labeling chain terminator molecules is that polymerases do not generally incorporate each terminator molecule with equal efficiency. Nucleotides or nucleosides associated with linker molecules in accordance with the methods of the present invention can be utilized in sequencing reactions to overcome this problem. Polymerases can incorporate nucleotides associated with linker moieties with equal efficiency. Detectable molecules, such as agent molecules linked to fluorescent dyes, or fluorescent molecules themselves, can be linked to the nucleotide

(or nucleoside)/linker molecules after the sequencing reaction to allow detection of the nucleic acid fragment. Nucleotide/linker moieties may be incorporated into the sequencing primers, or may be incorporated during the sequencing reaction, internally, or in the form of a chain terminator (for example, a dideoxy molecule associated with a linker). It is also contemplated that nucleotide/linker/ detectable moiety molecules (Morphatides) could be incorporated into an extending chain with acceptable efficiencies, thus have utility in sequencing reactions.

It will be appreciated to one skilled in the art that the methods of the present invention have several advantages over existing technologies. Among these advantages are the following: agent molecules and scaffolding molecules can be cycled separately or together; a variety of agent molecules and scaffolding molecules can be utilized; in many screening processes, reaction conditions (such as temperature or salt conditions) can be readily modified to enrich, screen, or select for particular association events; linker molecules can be utilized to generate novel secondary and tertiary structures; this is a cell free system, helping to avoid potential background contamination or false results.

In particular, if scaffolding molecules used are nucleic acids, the following advantages are afforded: single stranded, double stranded, triple stranded, or branched nucleic acids can be utilized; analogue nucleic acid molecules can be utilized, further increasing the potential structural diversity of the scaffolding molecule; linker molecules or components of linker molecules can be attached to multiple bases in the nucleic acid molecules; mixtures of bases can be utilized; sequencing can be employed to decipher desirable or resulting scaffolding molecules; mutations can be focused on subdomains within the nucleic acid molecules to modify the scaffolding molecule as desired, if so desired; modified polymerases can be employed for wider utility in the generation of

nucleic acid scaffolding molecules, and in the modification process, if this process requires the use of polymerases; and non-degradable nucleic acids can be utilized in this method.

These and other aspects of the present invention will be apparent to those skilled in the art.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXAMPLES

Example 1

Generation and Amplification of Linker Conjugated Scaffolding Molecules

1) Three pools of nucleic acid scaffolding molecules are generated using sequence randomized template nucleic acids generated (using solid state phosphoamidite chemistry followed by PCR amplification) with phenylboronic acid linker reagent conjugated dUTP molecules and dUTP, dATP, dCTP, and dGTP. Conjugated dUTP molecules are generated utilizing standard chemistry and phenylboronic acid linker complexing reagents. Different ratios of conjugated dUTP molecules to unconjugated dUTP molecules are present in each pool. Pool #1 contains conjugated:unconjugated dUTP present in a 1:10 ratio; pool #2 contains conjugated:unconjugated dUTP present in a 1:1 ratio; and pool #3 contains conjugated:unconjugated dUTP present in a 10:1 ratio. Error prone PCR is performed on each pool.

Generation of Morphatides

2) Five different conjugated amino acids (leucine, aspartic acid, glutamine, phenylalanine and tyrosine) (generated utilizing standard chemistry and phenylboronic acid complexing reagents) are combined to generate a mixture of conjugated agent molecules. Condensation reactions are performed utilizing the resulting mixture and each of the three pools, generating complexes of scaffolding molecules/linker molecules/agent molecules.

Selection and Evolution of Morphatides

3) Selection of thrombin binding Morphatides is performed on each pool as described infra in Example 4.

4) Scaffolding molecules are separated from agent molecules by reversing the linker via a small shift in pH and temperature, and cloned and sequenced as in Example 6.

5) Scaffolding molecules with the same or similar sequences as those obtained in Step 4 are generated via standard solid phase phosphoamidite chemistry, as in step 1, without the use of conjugated nucleotides.

6) Scaffolding molecules of selected Morphatides are then subjected to sexual PCR according to the following protocol to generate modified versions of the selected molecules:

a) Double stranded DNA from each pool and double stranded DNA from Step 6 are amplified, and free primers are removed from the samples;

b) About 5 µg of the DNA from each sample is digested with 0.15 units of DNAse I (Sigma, St. Louis, MO) in 100 µl of [50mM Tris-HCl ph 7.4, 1 mM $MgCl_2$], for 10-20 minutes at room temperature. The digested DNA is run on a 2% low melting point

agarose gel. Fragments of the desired size range are purified from the 2% low melting point agarose gel by electrophoresis onto DE81 ion exchange paper (Whatman, Hillsborougy, Oregon). The DNA fragments are then eluted from the paper with 1M NaCl and ethanol precipitated.

c) Purified fragments are resuspended at a concentration of 10-30 ng/µl in PCR Mix (0.2mM each dNTP, 22 mM $MgCl_2$, 50 mM KCl, 10mM Tris-HCl ph 9.0, 0.1 % Triton X-100, 0.3 µl Taq DNA polymerase, 50 µl total volume). A reassembly program of 94°C for 60 seconds, 30-45 cycles of [94°C for 30 seconds, 50-55° C for 30 seconds, 72°C for 30 seconds] and 5 minutes at 72°C is used in a thermocycler. Reaction can be followed by taking samples after 25, 30, 35, 40 and 45 cycles of reassembly.

Morphatide complexes are regenerated by complexing to agent molecules and new Morphatides are rescreened for binding to thrombin. Process is performed as many times as needed to narrow pool to most desirable Morphatides.

Example 2

Generation and Amplification of Scaffolding Molecules

Generation of Scaffolding Molecules

Four libraries of scaffolding molecules containing a random region (variable cores) and constant flanking regions are constructed. In each case, three of the four bases are incorporated with similar frequencies and one base is represented in a much reduced amount (e.g., $1/10_{th}$ of the other three bases, although this ratio depends upon several factors, including the length of the variable core), hereinafter the restricted or rare base. The restricted base later provides the template for the incorporation of the linker base or

nucleotide associated with linker molecules (a linker base can be a base analog as well). This approach permits the random modification of the variable cores in a manner that permits the position of the linker bases to be determined. This strategy is termed the 4x(3+1).

In a 4x(3+1) strategy, four pools of ssDNA oligomers with 72 nucleotides are prepared using solid state phosphoamidite chemistry. Eighteen pool specific base positions at the 5' and 3' end are kept constant for primer recognition during PCR. The central 36 positions are randomized by incorporating 3 bases at 31.3% each and one base, containing the linker, at 5%. After deprotection from the solid support, the pools are purified and recovered by precipitation using standard oligonucleotide purification protocols.

Therefore, scaffolding molecule sequences consist of three regions: 1) a fixed 5' sequence of 18 nucleotides, 2) a randomized middle part of 36 nucleotides, and 3) a fixed 3' sequence of 18 nucleotides. The two fixed sequences serve as PCR primer anchor sites. The variable core is synthesized as randomized sequences in four groups or pools. Variable cores in the first pool are synthesized with adenine reduced to 5% of the other bases, in the second pool with similarly reduced G, in the third pool with reduced % C, and in the fourth pool with reduced % T. These are termed the 3+1 reactions indicating that three of the bases are represented in equal amounts while each of the other nucleotides are present at the reduced level. In this manner, four pools of oligonucleotides are created. The rare base is the one that contains the linker molecule that can be connected or associated to agent molecules after each round of amplification-selection. It is the infrequent appearance of this base that reveals the potential sites of association or connection.

The two primer sequence anchors are designed as to not contain the nucleotide that is present in low concentrations in the variable core region. As a consequence, each pool will be anchored by pool specific primer pairs. Linker bases will thus only occur in the random core. This allows the determination of the positions of linker bases by DNA sequencing.

Amplification of Random Pools by PCR

The four pools are subjected individually to PCR amplification using two primers homologous to the anchored sequences. A selection protocol for single stranded DNA (ssDNA) is used. (Bock *et al.* 1992). These protocols are known in the art. One primer (the reverse primer, corresponding to the complement of the 3' end of the oligomer pools) is biotinylated to allow for later isolation of a single strand. For the rare base [that is present at low levels] in each of the four pools, a nucleotide is used that has the linker group attached. Hence, the rare nucleotides in the 36 nucleotide variable cores in the four pools of oligonucleotides have linker moieties associated or connected to them. The scaffolding molecules are then applied to a streptavidin-agarose column (equilibrated to 0.1 M Tris-HCl, pH 7.5, 0.1 M NaCl) (Griffin *et al.*, 1993; Bock *et al.*, 1992), and ssDNA (corresponding to original sequence library) eluted with 0.15 N NaOH. The flow through fraction (un-biotinylated strand) is collected, neutralized with acetic acid, concentrated and precipitated with ethanol.

Example 3

Generation of Morphatides

The rare DNA bases in the four random pools containing linkers are now individually reacted with the agent molecule threonine via a coupling reaction to generate the morphatides.

Selection of agent molecules to be used can depend on several factors. For example, aliphatic pentynyl groups can provide a site for hydrophobic interaction with a hydrophobic cluster of surface residues on thrombin. Knowledge of essential structures on the target molecule, such as catalytic site may be helpful in considering agent molecules. Of general use are charged groups such as several amino acids (Asp, Glu, and Arg) and hydrophobic moieties such as hydrophobic amino acids (Trp, Tyr, and Phe). However, many similar chemical groups are worthwhile considering. Small peptides are possible too.

Example 4

Selection of Morphatides with Affinity to Thrombin

After PCR amplification the four pools of morphatides are combined. Selection cycles are performed on concanavalin A column immobilized human thrombin (Bock *et al.* 1992). The DNA from Example 2 is precipitated and dissolved in selection buffer: (20 mM trig-acetate, pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$). The DNA is first applied to a concanavalin A-agarose column to remove those molecules that recognize concanavalin A or agarose structures. The flow through is then applied to a column containing thrombin bound to the concanavalin A - agarose support. The column is washed several times with selection buffer and the binding morphatides (selected morphatides) are eluted with 0.1 M α-methylmannoside, in the selection buffer (Griffin *et al.*, 1993; Bock *et al.*, 1992).

Example 5

Evolution of Selected Morphatides

Selected morphatides are subjected to phenol extraction to remove the thrombin. Scaffolding components are separated from the

threonine molecules. The resulting scaffolding molecules are precipitated with 20 µg glycogen and three volumes of ethanol. After resuspending the DNA, the oligomer solution is split into four pools, and amplified under error prone conditions. High fidelity amplification is utilized for the final amplification step, i.e. first sloppy PCR and then high fidelity PCR. For each pool the corresponding restricted nucleotide and the appropriate PCR primer sets are used. After PCR amplification the fragments are again reacted with the agent molecules. (Griffin; Bock) A total of six selection cycles over conA-agarose are performed. On average five cycles perform well.

Example 6

Cloning and Sequencing of the Scaffolding Components of High Affinity Morphatides

Characterization of the scaffolding components of the high affinity thrombin binding Morphatides after the final selection step is performed by cloning and sequencing of the nucleic acids. Single-stranded DNA (of the scaffolding components) is converted to dsDNA by PCR and cloned into an M13mp18 based TA modified cloning vector commercially available (Invitrogen Corp.). This vector also serves as a convenient repository for the generation of oligomers by PCR and generation of template for automated rapid DNA sequencing using robust M13 technology. This cloning and sequencing provides the DNA sequences of the selected scaffolding molecules, and, as only the rare nucleotide in each pool has the linker attached, also reveals the information about the position of the agent molecule. The sequencing results from the four random pools are analyzed using DNA alignment and phylogenetic software (widely available and utilized) to establish consensus sequences of the dominant binders.

Exampl 7

Characterization of Thrombin Specific Morphatides

Determination of Binding Characteristics

Binding constants to thrombin are determined. Because a considerable number of binding and competition experiments are performed, a rapid assay is employed. Thrombin is immobilized on microtiter plates (Tsiang *et al.* 1995b) and radioactively labeled Morphatides are added in series of different concentrations. After washing steps, the determination of binding constants is performed using rapid computerized phosphoimager technology. Washing steps are well known to one of ordinary skill in the art. Microtiter plates can be prepared in series and stored at 4°C for several weeks and the experiments performed using 96-well automated pipetting and washing steps. Crucial experiments to investigate cooperative binding effects, competitive binding and number of binding sites will be performed by equilibrium dialysis methods.

Example 8

Preparation of A Synthetic Oligonucleotide Reactive Phenylboronic Acid Complexing Reagent of General Formula XIX and Application Thereof (WO 95/20591, Phenylboronic Acid Complexes, August 3, 1995)

In the initial step of the synthesis 2-[2-(2-chloroethoxy]ethanol is condensed with N-hydroxy-phthalimide by refluxing in dimethylformamide containing one equivalent of triethylamine for 2 days. The product is precipitated by pouring into water, collected by filtration, washed with water, dried in a vacuum dessicator, and used without further purification.

In the second step of the synthesis, the crude product obtained above is refluxed briefly in a mixture of acetic acid and concentrated hydrochloric acid. After cooling, the precipitated phthalic acid is filtered from solution and the filtrate concentrated and then coevaporated repeatedly from small volumes of water to remove traces of acids. Finally, the aminooxy hydrochloride product is neutralized with $NaHCO_3$, extracted in ethyl acetate, dried over anhydrous $MgSO_4$, and concentrated in vacuo.

Synthetic oligonucleotides may be conjugated with a 2-cyanoethyl-N,N-diisopropylphosphoramidite phenylboronic acid complexing reagents, during the final step of an automated solid-phase oligonucleotide synthesis, to afford synthetic oligonucleotides having 5'- pendant phenylboronic acid complexing moieties.

Example 9

The following is a further example of the general methodology for the generation and screening of Morphatides.

General Outline of the Steps Performed:

1. Synthesis of degenerate single stranded DNA oligonucleotide

2. Chemical synthesis of DNA base derivative which serves as the "linker base"

3. Amplification of degenerate DNA oligonucleotide from step 1 by the polymerase chain reaction (PCR) including incorporation of the linker base during amplification

4. Generation of single stranded DNA from the PCR product using immobilized avidin carriers

5. Chemical coupling of agent molecules to the single stranded DNA from step 4 to generate pools of Morphatides consisting of oligonucleotide scaffolding components, linker components and agent molecules attached at the positions of the "linker base"

6. Immobilization of target molecules on solid support

7. Screening: selection of Morphatides with affinity to the immobilized target by mixing of the pool from step 5 with the immobilized target and washing away the unbound material

8. Evolution of scaffolding components of selected Morphatides (bound to target) by shuffling or sloppy PCR

9. Repeat steps 3 through 8 to select Morphatides with high affinity to the selected target molecules

Experimental Details:

1. Synthesis of degenerate single stranded DNA oligonucleotides (template to generate the scaffolds): Synthesis and purification of a degenerate oligonucleotide pool of the type A-Z-B is performed by standard methods (phosphoamidate chemistry, trityl off/on cartridge purification, HPLC, capillary electrophoresis). A denotes the following constant 18-mer 5' flanking region of the sequence: 5'AAA CCA GCA AAA ACA AAA3'; Z denotes a variable 34-mer core, called the "Morphacore" sequence, with A, C, G, and T occurring in variable but typically equal ratios at every position; and B denotes the following constant 18-mer 3' flanking region of the sequence: 5'AGA AAG AAA GAG CAA ACA3'

2. Chemical synthesis of DNA base derivatives which serve as the "linker base": Linker bases are synthesized using standard chemistry techniques. Phenylboronic acid derivatives of DNA bases

(phenylboronic acid dUTP) are commercially available from Prolinx, Bothell WA; SPDP (N-succinimidyl 3[pyridyldithio]propionate) derivatives of DNA (SPDP dUPT) bases can be synthesized commercially by Dalton Chemicals, Toronto ON, Canada.

3. Amplification of degenerate synthetic DNA oligonucleotide from step 1 by polymerase chain reaction (PCR) including incorporation of the linker base during the amplification reaction:

PCR is performed under the following conditions:
250 µM of each dATP, dGTP, dCTP
250 µM phenylboronic acid dUTP
0.5 µM forward primer 5'AAA CCA GCA AAA ACA AAA3'
0.5 µM reverse primer 5' biotin-TGT TTG CTC TTT CTT TCT3'.
5 mM tris(hydroxymethyl) aminomethane/HCl pH 9.0
60 mM potassium chloride
10mM magnesium chloride
2 ng/µl 70mer degenerate oligonucleotide from step 1

To generate DNA for a typical selection round, 5 µg of template should be used (~10^{14} molecules)
10 units of Taq polymerase per 100 µl assay volume

Amplification of DNA is performed in a thermocycler (PerkinElmer 2400) with the following program: 94°C for 1 min followed by 30 cycles of 94°C for 10 seconds, 55°C for 20 seconds and 72°C for 20 seconds, followed again by 72°C for 1 min and a 4°C hold. PCR products are analyzed on 2.5% PCR agarose gels (Invitrogen Corporation) and 10% polyacrylamide 7M urea gels (Novex, San Diego Ca) and purified and concentrated by standard 2 volume 100% ethanol precipitation followed a 70% ethanol wash. After drying and rehydration the DNA is redissolved in 10mM tris(hydroxymethyl) aminomethane/HCI, IµM ethylenediamine tetraaceticacid pH 8.0.

4. Generation of single stranded DNA from the PCR produce using immobilized avidin carriers: Single stranded Morphocore molecules are separated from the complement strands (reverse strand) by absorbing the biotinylated PCR product to Avidin agarose beads. The non biotinylated Morphacore strand is then melted off, and collected. Typically, 100µg of PCR product (from step 3) in 10 mM Tris(hydroxymethyl) aminomethane/HCI pH 8.0 (Tris pH 8.0) is batch bound to 500µl of avidin coupled resin in a 1 ml volume for 15 min at room temperature. This slurry is then transferred to a microcentrifuge spin column 7, and spun at 500xg for 1 min. The column is then washed with 2ml of 10mM Tris pH 8.0 by sequential resuspension of the resin in 1 ml, followed by centrifugation. The DNA single strands are eluted in a final volume of 1.8ml with 10-30mM NaOH by sequential resuspension of the resin in 0.9 ml, followed by centrifugation. The 0.9 ml elutions are neutralized by spinning into a collection tube containing 0.1ml 10mM Tris (pH unadjusted) and 10-30mM hydrochloric acid. Each fraction is analyzed by electrophoresis on a 10% polyacrylamide gel containing 7M urea (Novex), and visualized by ethidium bromide staining.

5. Chemical coupling of agent molecules to the single stranded DNA from step 4 to generate pools of Morphatides consisting of oligonucleotide scaffolding components, linker components and agent molecules attached at the positions of the "linker base": Single stranded DNA with phenylboronic acid covalently linked to DNA bases generated in step 4 is used as a substrate for the coupling of agent molecules. Agent molecules are groups (for example amino acids) which are covalently linked to salicyl hydroxamic acid functional groups. The salicyl hydroxamic acid will form a reversible complex with the phenylboronic acid moieties of the linker bases in the DNA. Coupling is performed in 0.1 M sodium bicarbonate pH 9.7 at 37°C for 24 hours. Concentration of the reactants is 1 mM of the salicyl hyroxamic acid derivative of the agent molecule and 100 µg per ml of single stranded DNA from step

4. After the incubation period, the coupling reaction is purified by commercially available SNAP purification kits (Invitrogen Corporation, Carlsbad, CA) which separate the reaction product (the Morphatides) from the unreacted salicyl hydroxamic acid monomeric binder molecules. Morphatides are eluted from the spin columns, ethanol precipitated and resuspended in the selection buffer used in step 7 below.

6. Generation of Immobilized targets.

Polypeptide target molecules are covalently attached to 3.76 µM carboxyl magnetic particles 1 via a two step coupling procedure2 using the carbodiimide EDAC [1-ethyl-3-(3 diethylaminopropyl) carbodiimide hydrochloride]3 and sulfoNHS (N-Hydroxysulfosuccinimide) 4method. For a typical selection $1x10^9$ beads are reacted in 10ml 50mM MES (2-[NMorpholino]ethanesulfonic acid), 500mM NaCl, pH6.0 containing 2mM EDAC, and 5mM sulfoNHS for 15 min at room temperature to allow formation of the sulfoNHS activated ester intermediate. The beads are then washed 3 times with 10 ml coupling buffer (50 mM potassium phosphate pH 7.5), resuspended in 10 ml coupling buffer containing 10µM target molecules, and allowed to react for 2 hours at room temperature. The beads are then washed 3 times with 10 ml coupling buffer and reacted in coupling buffer containing 200µM ethanolamine for two hours at room temperature to block any remaining reactive groups. The reacted and blocked beads are then washed 6 times in 10 ml coupling buffer and stored at 4°C at a concentration of $1x10^8$ beads/ml in coupling buffer. Control beads are prepared in the same manner as above, substituting ethanolamine for the target molecule.

To estimate the number of target molecules coupled to the beads, a Bichinconic acid protein assay 5 is performed on $1x10^7$ beads. The supernatant from the bead/assay mixture is read at 570 nm and

compared to a standard curve to calculate the quantity of protein coupled to the beads. Given the molecular weight of the coupled polypeptide the number of molecules per bead is calculated. Typical coupling efficiencies with this protocol are 10^6 molecules coupled per magnetic latex bead.

7. Selection of Morphatides with affinity to the immobilized target by mixing of the pool from step 5 with the immobilized target and washing away the unbound material:
The coupled Morphatide pool from step 5 is used for selection of target molecules coupled to magnetic beads as described in step 6. The selection buffer used is 20 mM Tris/acetate pH 7.2, 140 mM NaCl. The number of latex beads coupled with target molecules used in the first round of selection is calculated to provide one target molecule per Morphatide sequence represented in the pool. Thus at a coupling efficiency of 10^6 molecules of target per magnetic latex bead and a library diversity of 10^{14} molecules, 10^8 beads are used in the first round of selection. As a first step, Morphatides which bind unspecifically to the carrier are removed from the pool by adding 10^8 control coupled beads to the pool. The mixture is incubated at room temperature for 4 hours with gentle shaking, the beads are collected at the side of the tube with a suitable magnet and the supernatant containing unbound Morphatides is removed. This Morphatide pool is added to the coupled latex beads (0.5 ml) and incubated at room temperature for 16 hours with gentle shaking (platform shaker). After this binding step the latex beads are washed five times with 2 ml of selection buffer, again using a magnet to collect the beads at the side of the tube. The supernatants from the washing steps are combined in a tube and latex beads are collected at the side of the tube, washed, and combined with the bulk of the beads to avoid losses during the washing steps. Morphatide molecules bound to targets are released by resuspending the beads in 2% sodium dodecyleulfate and heating to 90°C for 10 min. After collecting the beads the supernatant is

extracted with phenol/chloroform and ethanol precipitated. The pellet after ethanol precipitation is resuspended in 20 µl of 10 mM Tris/HCI pH 8.5 and used as template in a sloppy amplification reaction or shuffling reaction as per step 8 below.

8. Evolution of scaffolding components of Morphatides bound to target by shuffling or sloppy PCR Morphatides released from the target in step 7 are separated into scaffolding components and agent molecules, and the scaffolding components are subjected to amplification "sloppy PCR" reaction for evolution. Scaffolding components can also be "shuffled" by suspending the fragments in a PCR mix (0.2 mM each dNTP, 2.2 mM $MgCl_2$, 50mM KC1, 10mM Tris-HCI pH 9.0, 0.1% Triton X-100), at a concentration of 10-30ng/µl and amplifying under the following conditions: 94°C for 60 seconds, 40 acycles of 94°C for 30 seconds, 50-55°C for 30 seconds, 72°C for 30 seconds followed by a 5 minute incubation at 72°C in any standard thermocycler.

9. Repeat steps 3 through 8 to select Morphatides with high affinity to the selected target molecules. In subsequent rounds of selection the same methods are employed as described above. However, in order to introduce moderate selective pressure into the rounds of selection, the number of target molecules coupled to magnetic latex beads is reduced by a factor of two for each selection rounds, thus in a typical series of experiments with 8 round of selection the number of targets is reduced by a factor of 128 (the complete process to select Morphatides using this method is termed exponential morphatide selection and evolution, EMSE). To follow the progress of the selection, Morphatides released from the target after round two are routinely cloned and sequenced. Typically double stranded linear DNA after the two stage PCR is cloned into a topoisomerase clogging vector (Topo 1, Invitrogen) with the capacity to allow direct selection for inserts. 30 independent clones are isolated and sequenced per selection round.

1. Spherotech Inc. Libertyville, Illinois cat no. CM-30-50

2. Protocol adapted from GT Hanson, Bioconjugate Techniques, Academic press inc. 1996, pp 176.

3. Sigma chemical company, St. Louis, Missouri, cat no. E 1769

4. Pierce Chemical Company, Rockford, Illinois, cat no. 24510

5. Pierce Chemical Company, Rockford, Illinois, cat no. 23223

6. Vector Laboratories, Burlingame, California, cat no. A-2010

7. Costar Corp., Cambridge, Massachusetts cat no. 8170

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